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Research Article

Four new species of *Trichoderma* (Hypocreaceae, Hypocreales) discovered in the staple food bamboo of pandas

Feihu Wang[1,2,3], Xiulan Xu[4], Feng Liu[1,2,3], Shasha Xiang[1,2,3], Xinyue Li[1,2,3], Yinggao Liu[1,2,3], Chunlin Yang[1,2,3]

1 *College of Forestry, Sichuan Agricultural University, Chengdu, Sichuan, 611130, China*
2 *Forest Ecology and Conservation in the Upper Reaches of the Yangtze River Key Laboratory of Sichuan Province, Chengdu 611130, Sichuan Province, China*
3 *Sichuan Mt. Emei Forest Ecosystem National Observation and Research Station, Chengdu 611130, Sichuan Province, China*
4 *Forestry Research Institute, Chengdu Academy of Agricultural and Forestry Sciences, Chengdu 611130, Sichuan Province, China*

Corresponding authors: Yinggao Liu (lyg927@263.net); Chunlin Yang (yangcl0121@163.com)

Abstract

Trichoderma fungi are significant saprophytic resources in nature, with only a minority of species documented as pathogenic fungi. Due to their widespread distribution, this genus of fungi has attracted considerable attention in recent years. During an investigation of fungal resources within the staple food bamboo species for giant pandas in China conducted from 2023 to 2024, a high diversity of *Trichoderma* species was observed. In this study, eight collected specimens were compared morphologically with known species, and DNA sequence analysis was performed using a multi-gene (ITS, *tef*1-α, and *rpb*2) dataset to establish phylogenetic relationships, ultimately leading to the identification of four *Trichoderma* species. The research uncovered four novel *Trichoderma* species: *Trichoderma bashania*, *T. fargesia*, *T. mianyangensis* and *T. yaanensis*. Phylogenetic analysis revealed that each of these new species forms a distinct lineage, with *Trichoderma bashania*, *T. fargesia*, *T. mianyangensis* and *T. yaanensis* all belonging to the Koningii section. All these newly identified species were isolated from the litter of the staple food bamboo species for giant pandas. This study provides morphological descriptions and illustrations of these four new species, along with DNA phylogenetic relationships based on the analysis of the multi-gene dataset. The findings indicate that *Trichoderma* fungi are widely present in the ecosystem of the staple food bamboo species for giant pandas and warrant close attention.

Key words: Morphology, multi-gene, pandas, phylogenetic analyses, staple food bamboo, taxonomy, *Trichoderma*

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Introduction

The giant panda (*Ailuropoda melanoleuca*), an endemic and rare species in China, is hailed as a "living fossil" and "China's national treasure". It also serves as the ambassador for the World-Wide Fund for Nature (WWF) and is a flagship species for global biodiversity conservation, as well as one of the most beloved wild animals worldwide (Ran et al. 2009). Although giant pandas are classified as carnivores and still retain the digestive system of carnivores, their diet is extremely narrow, with bamboo constituting the vast majority of their

food intake. As a highly specialized species in terms of diet, bamboo resources account for over 99% of their food resources (Zhang et al. 2014). Based on fossil records, giant pandas have been exclusively bamboo-eaters for at least 2 million years. Scientists have never ceased their research into how giant pandas have adapted to a bamboo diet (Hansen et al. 2010; Senshu et al. 2014; He et al. 2020). However, not all bamboo species in nature can serve as food for giant pandas, and this singular food source makes giant pandas particularly vulnerable. Therefore, the study of the staple bamboo species for giant pandas is warranted (Tian et al. 2019). Currently, research on the staple bamboo species for giant pandas is limited to nutritional component analysis, while studies on microorganisms within the ecosystem of staple bamboo species remain largely unexplored (Helander et al. 2013; Jin et al. 2020). Research indicates that most plants are surrounded by a stable and complex microbial community, and changes in these microorganisms may trigger alterations in plants' growth, development, health status, stress resistance, and other aspects. (Shang et al. 2024; Yang et al. 2024). Consequently, studying the fungi associated with the staple bamboo species for giant pandas is of great importance.

The genus *Trichoderma* (Hypocreaceae, Hypocreales, Sordariomycetes) was originally established by Persson in 1794. *Trichoderma* species are saprophytic fungi, commonly found in soil, wood, and litter (Hasan et al. 2012; Abo-Elyousr et al. 2014; Poveda et al. 2019; Zhao et al. 2023). Additionally, some *Trichoderma* species are significant pathogenic fungi, serving as key pathogens causing green mold disease and leading to the decay of a large number of cultivated mushrooms (Savoie and Mata 2003; Komon-Zelazowska et al. 2007; Tarafder et al. 2024). Finally, an increasing body of research indicates that *Trichoderma* species also function as important plant endophytes, playing crucial roles in the growth and development of plants (Lorito et al. 2010; Harman et al. 2021). Consequently, due to their economic and ecological significance, *Trichoderma* has evolved into a genus with a rich diversity of species. Currently, more than 500 species have been reported and recognized (http://www.indexfungorum.org). Meanwhile, species within this genus have been widely utilized, exerting a profound impact on humanity. They are efficient producers of bio-enzymes, important sources of biocontrol agents against plant pathogens, and key participants in environmental remediation, thus possessing extremely high economic value (Tripathi et al. 2013; Abdel-Mageed et al. 2022; Zhang et al. 2023).

Initially, the identification of *Trichoderma* relied solely on the different colors of ascospores in its sexual stage for differentiation (Rifai 1969). During its sexual stage, *Trichoderma* can produce ascospores of two distinct colors: hyaline (transparent) and green. Early research was based on these two-color variations for classification purposes (Bissett 1991). Chaverri and Samuels (2004) took the lead in conducting a comprehensive study on *Trichoderma* species with green ascospores. Although this provided early insights into the study of *Trichoderma*, the accuracy of identification was far from sufficient. Subsequently, Jaklitsch and Voglmayr (2015a) proposed an integrated classification system primarily based on molecular phylogenetic analysis rather than ascospore color, dividing the genus into six subclades: the Ceratocladosporium subclade, the Viride subclade, the Harzianum subclade, the Helicum subclade, the Asperellum subclade, and the Strictipile subclade. Currently, the identification of *Trichoderma* is gradually becoming standardized. The widespread use

of DNA barcoding, such as the *rpb*2 and *tef*1-α coding genes, for identification purposes has enhanced the accuracy and standardization of the process (Cai and Druzhinina 2021; Zheng et al. 2021).

Species of the genus *Trichoderma* are extensively distributed across most regions worldwide, with their survival range spanning high-latitude areas from the far north to the far south. They demonstrate remarkable ecological adaptability, with their presence traced in ecosystems ranging from humid tropical and subtropical rainforests to arid deserts, temperate grasslands, and even northern boreal ecosystems (Cai and Druzhinina 2021). The rich biodiversity exhibited by this group, along with the continuous discovery and exploration of novel resources within it, has successfully garnered the attention of numerous researchers globally. Similarly, China has also shown a high level of interest in this group. However, in previous studies, most species were isolated from soil, and the majority of research was reported based on cultured strains (Zhang et al. 2022; Zhao et al. 2021, 2025). In this study, three sexual morph specimens and one asexual morph specimen were collected from four staple bamboo species for giant pandas, namely *Bashania faberi*, *Fargesia qinlingensis*, *Phyllostachys sulphurea*, and *Bambusa emeiensis*. The phylogeny of the four species were reconstructed using the gene sequences of ITS, *tef*1-α, and *rpb*2. The results met the criteria for identifying new species of *Trichoderma* (Cai and Druzhinina 2021).

Materials and methods

Specimen collection and isolation

This study involved collecting litter samples from the staple bamboo species for giant pandas in China (Sichuan Province and Shaanxi Province) between 2023 and 2024. The samples were collected using plastic sealing bags and transported back to the laboratory for subsequent experiments. The samples were examined and sectioned using a Leica stereo microscope (EZ 4). Single spore isolation was performed following the method described by Chomnunti et al. (2014). Hand-sectioning techniques were employed to prepare slide specimens of the fruiting bodies. Morphological structures were photographed using an Olympus BX53 microscope. All measurements of structural components were conducted using Tarosoft Image Framework software (version IFW 0.97). The type specimens have been deposited at the Herbarium of Sichuan Agricultural University (SICAU) in Wenjiang, Sichuan, China. All live cultures are preserved at the Strain Collection of Sichuan Agricultural University (SICAUCC). Based on the method provided by Jayasiri et al. (2015) for obtaining facial login numbers (http://www.facesoffungi.org/), all strains in this study have been registered in the Fungi Index (2025, http://www.indexfungorum.org/).

DNA extraction, PCR amplification and nucleotide sequencing

Using a sterile stainless-steel spoon, fungal hyphae were scraped from Potato Dextrose Agar (PDA) and placed into a centrifuge tube containing steel beads. The hyphae were then thoroughly ground using a freeze-grinding in-

strument. The ground fungal hyphae were subjected to total genomic DNA extraction using a novel rapid extraction kit for plant genomic DNA (Aidilai Biotechnology Company, China). Three genetic regions were amplified, namely the Internal Transcribed Spacer (ITS) rRNA, Translation Elongation Factor 1-alpha (*tef*1-α), and RNA polymerase II subunit (*rpb*2). The amplified genetic markers and their corresponding primers are listed in (Table 1). Polymerase Chain Reaction (PCR) was performed in a 25 μL reaction mixture containing 12.5 μL of MasterMix (Beijing, China), 9.5 μL of deionized water 1 μL of DNA template, and 1 μL of each forward and reverse primer. The amplification conditions are shown in (Table 2). The PCR products were sequenced by Qingke Biotechnology Co., Ltd. in China. The newly generated sequences have been deposited in GenBank (Table 3).

Sequence alignment and phylogenetic analyses

A phylogenetic analysis of the genus *Trichoderma* was conducted based on ITS, *tef*1-α, and *rpb*2 sequence data. In this study, the strain *T. hamatum* (DAOM 167057) and *T. asperellum* (GJS 04-217) were selected as the outgroup for phylogenetic analysis. The sequences generated in this study were examined and assembled using BioEdit v.7.0.9 (Hall 1999). Sequence alignment was performed using MAFFT v.7 online software (https://mafft.cbrc.jp/alignment/server/) (Katoh and Standley 2013). Based on the combined dataset, phylogenetic analyses were conducted using Maximum Likelihood (ML) and Bayesian Inference (BI), were constructed as described in Zhao et al. (2025). The phylogenetic trees were viewed using FigTree v1.4.3 (Rambaut and Drummond 2016) and edited using Adobe Illustrator 2021 (version 2.6.0.44) and Adobe Photoshop CS6 software (Adobe Systems, USA).

Table 1. Sequences of primers used in this study.

Gene markers	Primers	Sequences of Primers 5'-3'	References
ITS	ITS5	GGAAGTAAAAGTCGTAACAAGG	White et al. 1990
	ITS4	TCCTCCGCTTATTGATATGC	
*tef*1-α	728F	CATCGAGAAGTTCGAGAAGG	Carbone and Kohn 1999
	EF2	GGARGTACCAGTSATCATG	O'Donnell et al. 1998
*rpb*2	5F	GAYGAYMGWGATCAYTTYGG	Liu et al. 1999
	7CR	CCCATRGCTTGYTTRCCCAT	

Table 2. Primers and PCR protocols used in this study.

Gene markers	Primers	Optimized PCR Protocols	References
ITS	ITS5	94 °C 3 min; 35 cycles of 94 °C 30 s, 55 °C 50 s, 72 °C 1 min; 72 °C 10 min; 4 °C on hold	White et al. 1990
	ITS4		
*tef*1	728F	94 °C 3 min; 35 cycles of 94 °C 30 s, 55 °C 50 s, 72 °C 1 min; 72 °C 10 min; 4 °C on hold	Carbone and Kohn 1999
	EF2		
*rpb*2	5F	95 °C 5 min; 35 cycles of 95 °C 1 min, 52 °C 2 min, 72 °C 90 s; 72 °C 10 min; 4 °C on hold	Liu et al. 1999
	7CR		

Table 3. Specimen information and GenBank accession numbers of the sequences used in this study.

Species	Strain	GenBank Accession Numbers		
		ITS	*rpb2*	*tef1α*
Trichoderma albofulvopsis W.T. Qin & W.Y. Zhuang	9930T	/	KU529138	KU529127
Trichoderma amoenum Z.F. Yu & Y.F. Lv	YMF 1.06209T	/	MT052192	MT070146
Trichoderma arenarium F. Cai, M.Y. Ding & Druzhin	TUCIM 10301T	/	MT242310	MT242303
Trichoderma asperellum Samuels, Lieckf. & Nirenberg	GJS 04-217	DQ381957	DQ333564	DQ381958
Trichoderma atroviride P. Karst	GJS 02-134	DQ315466	/	DQ307547
Trichoderma austrokoningii Samuels & Druzhin	GJS 99-146T	DQ323423	DQ367716	DQ307561
***Trichoderma bashania* Feihu Wang & C.L. Yang**	**SICAU 25-0179**	**PV789471**	**PV828316**	**PV828324**
***Trichoderma bashania* Feihu Wang & C.L. Yang**	**SICAU 25-0180T**	**PV789472**	**PV828317**	**PV828325**
Trichoderma caribbaeum Samuels & Schroers	GJS 97-3T	DQ313131	DQ328607	DQ284977
Trichoderma changiae Y.H. Wei & S.S. Tzean	BCRC 24F0002T	PP265989	PP273695	PP273697
Trichoderma dingleyae Samuels & Dodd	GJS 02-50T	DQ333548	DQ367718	DQ284978
Trichoderma dorothopsis A.A. Tomah & J.Z. Zhang	HZA5ET	/	MH647795	MK850827
Trichoderma erinaceum Bissett, C.P. Kubicek & Szakács	DIS 8	DQ313147	DQ323450	DQ284970
***Trichoderma fargesia* Feihu Wang & C.L. Yang**	**SICAU 25-0185T**	**PV789477**	**PV828320**	**PV828328**
***Trichoderma fargesia* Feihu Wang & C.L. Yang**	**SICAU 25-0186**	**PV789478**	**PV828321**	**PV828329**
Trichoderma hamatum (Bonord.) Bainier	DAOM 167057T	Z48816	DQ111962	AF456911
Trichoderma hongkuii C.L. Zhang	GDMCC 3.1017T	/	OR779477	OR779504
Trichoderma istrianum Jaklitsch & Voglmayr	CBS 130539T	/	KJ665281	KJ665523
Trichoderma intricatum Samuels & Dodd	GJS 97-88T	AY380913	AY376684	AY376060
Trichoderma koningii Oudem	CBS 457.96T	Z79628	DQ341180	AF456909
Trichoderma koningiopsis Samuels, Carm. Suárez & H.C. Evans	GJS 93-20	/	DQ381954	DQ284966
Trichoderma merleae merleae Y.P. Tan, Minns, Valter, Marney & E. Lacey	MST FP3586T	PQ570877	PQ572711	PQ572712
***Trichoderma mianyangensis* Feihu Wang & C.L. Yang**	**SICAU 25-0183T**	**PV789475**	**PV828322**	**PV828330**
***Trichoderma mianyangensis* Feihu Wang & C.L. Yang**	**SICAU 25-0184**	**PV789476**	**PV828323**	**PV828331**
Trichoderma neohongkuii C.L. Zhang	GDMCC 3.1018T	/	OR779481	OR779508
Trichoderma ovalisporum Samuels & Schroers	DIS 70aT	/	AY376671	AY376037
Trichoderma parahamatum C.L. Zhang	GDMCC 3.1020T	/	OR779474	OR779501
Trichoderma parahongkuii C.L. Zhang	GDMCC 3.1019T	/	OR779476	OR779503
Trichoderma petersenii Samuels, Dodd & Schroers	GJS 04-355T	/	DQ333570	DQ284980
Trichoderma rogersonii Samuels	GJS 04-158T	/	DQ333567	DQ307563
Trichoderma stilbohypoxyli Samuels & Schroers	CBS 992.97T	/	DQ111967	DQ109546
Trichoderma taiwanense Samuels & M.L. Wu	GJS 95-93T	DQ313141	/	DQ284973
Trichoderma viride Pers	CBS 433.34	AF456922	/	AF456905
Trichoderma gamsii Samuels & Druzhin	GJS 04-09	DQ315459	/	DQ307541
Trichoderma vinosum Samuels	GJS 02-54	DQ315447	/	DQ307528
Trichoderma viride Pers	GJS 04-353	DQ323418	/	DQ307551
Trichoderma texanum Q.V. Montoya, L.A. Meirelles, P. Chaverri & A. Rodrigues	LESF551T	HQ608136	KT278920	KT278988
Trichoderma tibetica Z.F. Yu & X. Du	YMF 1.05583T	MK779177	MK779178	MK779179
***Trichoderma yaanensis* Feihu Wang & C.L. Yang**	**SICAU 25-0181T**	**PV789473**	**PV828318**	**PV828326**
***Trichoderma yaanensis* Feihu Wang & C.L. Yang**	**SICAU 25-0182**	**PV789474**	**PV828319**	**PV828327**

Notes: superscript T represents ex-type or ex-epitype isolates. "/" means that the sequence is missing or unavailable. The new sequence is displayed in bold red.

Genealogical concordance phylogenetic species recognition analysis

Genealogical Concordance Phylogenetic Species Recognition (GCPSR) is a pivotal and unique method in the field of biological research for model validation. It can efficiently and precisely detect significant recombination events within datasets (Taylor et al. 2000). In this study, we employed the powerful SplitsTree V4 software and conducted an in-depth analysis of the data using the pairwise homoplasy index (PHI) test. This test meticulously examines genetic similarities, enabling us to accurately determine the recombination levels among closely related species. When the pairwise homoplasy index falls below the threshold of 0.05 ($\Phi w < 0.05$), it clearly indicates the presence of significant recombination events in the dataset. To ensure the accuracy and comprehensiveness of the analysis results, the study selected a concatenated multi-gene dataset encompassing all closely related species. This comprehensive dataset provides a more reliable genetic foundation for the research. Based on this dataset, a split graph was constructed using the LogDet transformation and split decomposition options to visually present the complex genetic relationships.

Results

Phylogenetic analyses

This dataset comprises composite sequences formed by concatenating ITS, *tef*1-α and *rpb*2 gene sequences. These sequences are employed to elucidate and determine the phylogenetic position of the new species within the genus *Trichoderma*. The phylogenetic tree was performed for placement determination. 41 strains were included in the combined analyses, which comprised 3705 characters (1831 characters for *tef*1-α, 672 characters for ITS and 1202 characters for *rpb*2, including alignment gaps). The tree was rooted with *T. hamatum* DAOM 167057 and *T. asperellum* GJS 04-217. The best scoring RAxML tree had a fnal likelihood value of -16267.318033. The matrix had 1213 distinct alignment patterns, with 47.89% of undetermined characters and gaps. Estimated base frequencies were: A = 0.228333, C = 0.285248, G = 0.235065, T = 0.251354; substitution rates AC = 1.085759, AG = 2.939729, AT = 1.067672, CG = 0.871080, CT = 4.506612, GT = 1.000000; gamma distribution shape parameter α = 0.458789. The Bayesian inference analyses was implemented by MrBayes v.3.2.2 with the best-fit model (HKY+I+G for tef1-α; GTR+I+G for ITS and rpb2) of evolution estimated with MrModeltest 2.2. The maximum likelihood (ML) and Bayesian methods (BI) for phylogenetic analyses resulted in trees with similar topologies, and the result of ML analysis is shown in Fig. 1.

Taxonomy

Trichoderma bashania **Feihu Wang & C.L. Yang, sp. nov.**
Index Fungorum number: IF904040
Fig. 3

Etymology. Named after the genus of the host plant from which the holotype was collected, *Bashania faberi*.

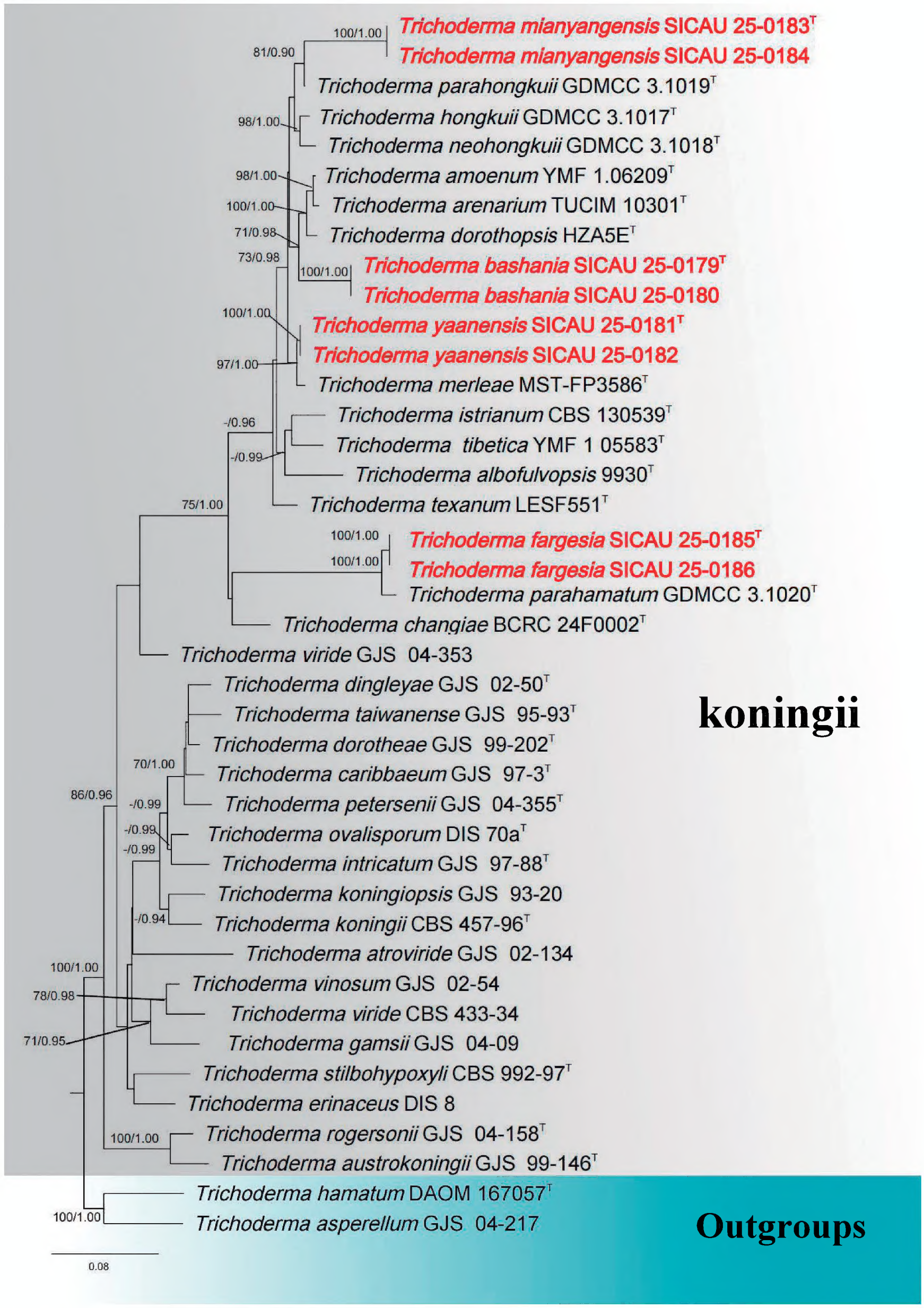

Figure 1. Phylogram generated from maximum likelihood analysis based on combined ITS, *tef*1-α and *rpb*2 sequence data of *Trichoderma* (koningii species complex) taxa. The species determined in this study are indicated in bold red. Bootstrap values (BS) from maximum likelihood (MLBS, left) higher than 70 BS and Bayesian posterior probabilities (BYPP, right) greater than 0.90 are given at the nodes. Hyphens (-) represent support values less than 70 MLBS/0.90 BYPP. The ex-type strains are in T.

Holotype. SICAU 25-0179.

Habitat. On the culm of *Bashania faberi*.

Description. ***Sexual morph***: ***Stromata*** scattered, sparsely spreading, growing on the side adjacent to the ground surface, base narrow, pulvinate or discoidal. Color of stromata pale orange-yellow when fresh, brown when mature, with a diameter of 1–4 mm and a thickness of 1–2 mm. Immature, surface of stromata finely velvety, upon maturity, tuberculate with fine granules or slightly rugose. Outline circular, oblong or irregularly lobed. Surface smooth, tubercular or rugose, when young finely velvety. ***Ascomata*** 120–190 × 80–140 µm ($\bar{x}$ = 145 × 115 µm, n = 30), numerous, with 10–30 ascomata present, and are sub-globose or pear-shaped in form. ***Ostioles*** flush with the surface, 18–30 µm wide at the apex, 26–38 µm high (n = 20). ***Peridium*** 13–24 µm (n = 60) thick at the base, composed of hyaline textura globosa. ***Asci*** 60–90 × 3.5–6 µm ($\bar{x}$ = 85 × 4.5 µm, n = 30), short stipe, containing 13-ascospores, apex not thickened, hyaline, cylindrical. ***Ascospores*** 3–6 × 2.5–4.5 µm ($\bar{x}$ = 4.5 × 3 µm, n = 50), hyaline, containing 1–2 oil droplets, single-celled, non-septate, and sub-globose. ***Asexual morph***: ***Conidiophores*** simple structure, with 1–3 solitary phialides borne at the tips of lateral branches. ***Phialides*** measure 4–18 × 1.5–3 µm ($\bar{x}$ = 14 × 2.5 µm, n = 20), mostly lageniform, less commonly subfusiform, and typically do not thicken near the base. ***Conidia*** vary in size and shape, measuring 2.5–4.5 × 2–4 µm ($\bar{x}$ = 4 × 3 µm, n = 50), oval, ellipsoid, and hyaline, smooth-surfaced.

Material examined. China • Sichuan Province, Chengdu City, Dujiangyan, Primitive Forest of *Bashania faberi* (31°12'8.63"N, 103°42'49.96"E, Alt. 1212 m), 11 December 2023, Feihu Wang, WFH202312013, (SICAU 25-0179, holotype), ex-type culture SICAUCC 25-0151. *ibid*. WFH202312013B (SICAU 25-0180, paratype), living culture SICAUCC 25-0152.

GenBank accession numbers. SICAUCC 25-0151 (ITS: PV789471; *tef*1-α: PV828324; *rpb2*: PV828316); SICAUCC 25-0152 (ITS: PV789472; *tef*1-α: PV828325; *rpb2*: PV828317).

Culture characters. This fungal strain exhibits optimal growth on all tested media at 30 °C, whereas growth is restricted or completely inhibited at 35 °C. On PDA, at 30 °C, the colony slowly covers a 60-mm Petri dish within 10 days, displaying a grayish-white color with a radial growth pattern and fluffy texture. Conidiation initiates after 15 days, producing numerous white conidia that aggregate into irregularly margined patches. On SNA, growth is the slowest among the three media; at 30 °C, the colony reaches a radius of 50 mm after 10 days, remaining white with a radial pattern and sparse hyphae. Conidiation begins 15 days post-inoculation, forming white, irregularly margined conidial patches composed of aggregated conidia. No odor or pigment diffusion is detected. On MEA, at 30 °C, the colony rapidly covers the entire 60-mm dish within 7 days, featuring a well-defined border, radial growth, and creamy-white mycelium with dense, abundant aerial hyphae. No distinct odor or diffusing pigment is observed.

Notes. Phylogenetically, *Trichoderma bashania* (SICAU 25-0179) and (SICAU 25-0179) formed a distinct clade and is related to *T. dorothopsis* (HZA5E) in the Koningii clade, but the similarities of *rpb*2 and *tef*1-α between these two species were only 95.3% and 98.3%, respectively. From a morphological perspective, only the asexual stage of *T. dorothopsis* has been described, and there are differences in conidia between these two *Trichoderma* species. The conidia of *T. bashania* are elliptical or ovoid, whereas those of *T. dorothopsis* are globose to subglobose

(Tomah et al. 2020). Phylogenetic analysis indicates that the new taxon *T. bashania* (SICAU 25-0179) is closely related to *T. dorothopsis* (HZA5E) (Fig. 1). However, our strain exhibits nucleotide differences from *T. dorothopsis* in the *rpb*2 region amounting to 4.7% (39/822, 3 gaps), 1.7% (13/753, 0 gap) differences in *tef*1-α. The PHI test revealed no significant recombination event between our strain and the closely related taxa (Φw = 1.00) (Fig. 2). These differences also support the classification of *T. bashania* as a distinct species separate from *T. dorothopsis*.

Trichoderma fargesia **Feihu Wang & C.L. Yang, sp. nov.**

Index Fungorum number: IF904041

Fig. 4

Etymology. Named after the genus of the host plant from which the holotype was collected, *Fargesia qinlingensis*.

Holotype. SICAU 25-0185.

Habitat. On the culm of *Fargesia qinlingensis*.

Description. ***Sexual morph***: ***Stromata*** scattered or aggregated in small numbers, lenticular to pulvinate in shape. Centrally attached, free margins, rounded, angular, or irregular in outline. Color ranges from light reddish-brown to dark reddish-brown,

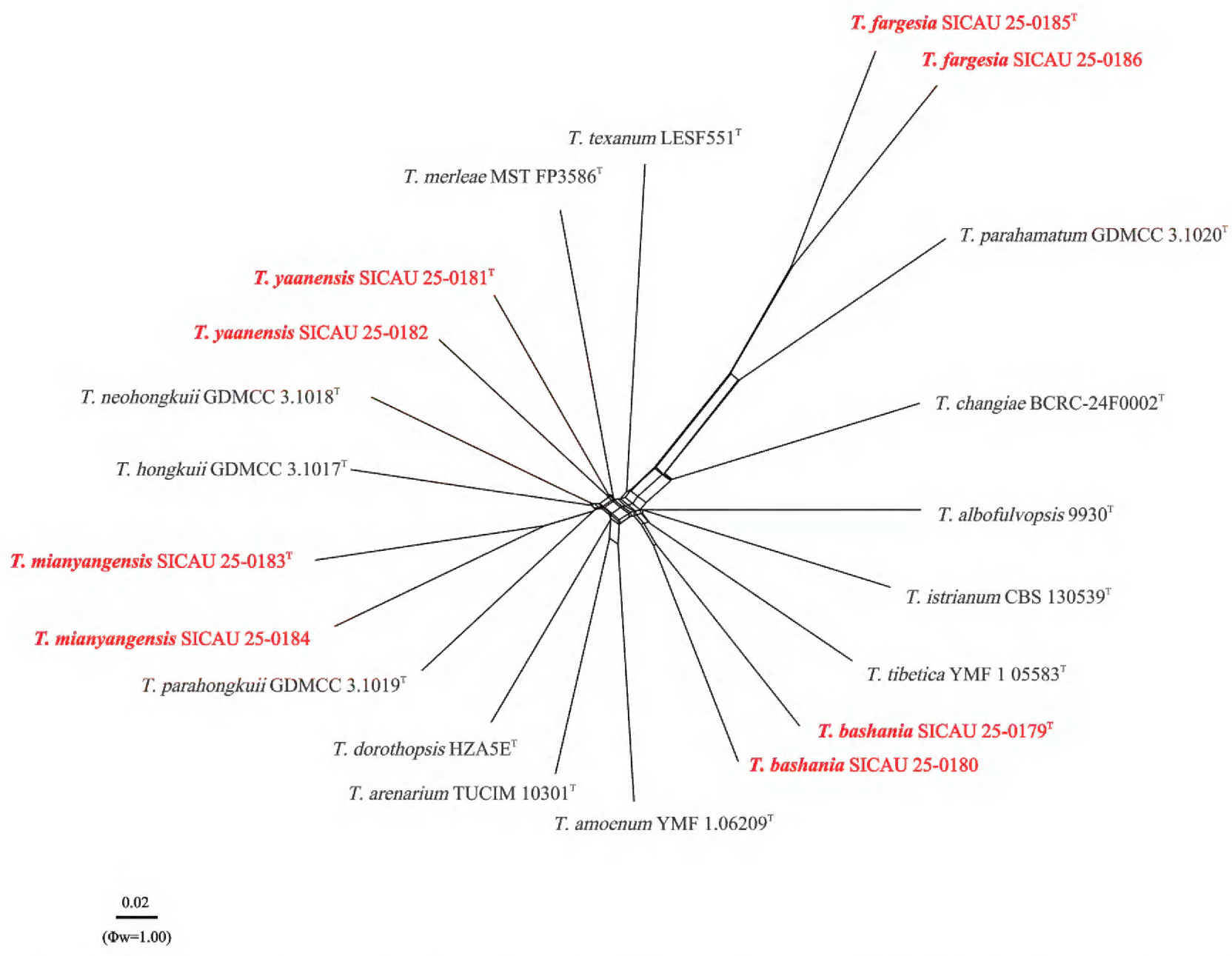

Figure 2. The PHI test results for *Trichoderma bashania*, *T. fargesia*, *T. mianyangensis*, *T. yaanensis*, and closely related species were obtained using both LogDet transformation and splits decomposition methods. The PHI test did not find any statistically significant recombination (Φw = 1) in the data set. The new strains are represented in red and bold. "T" represents the type strain.

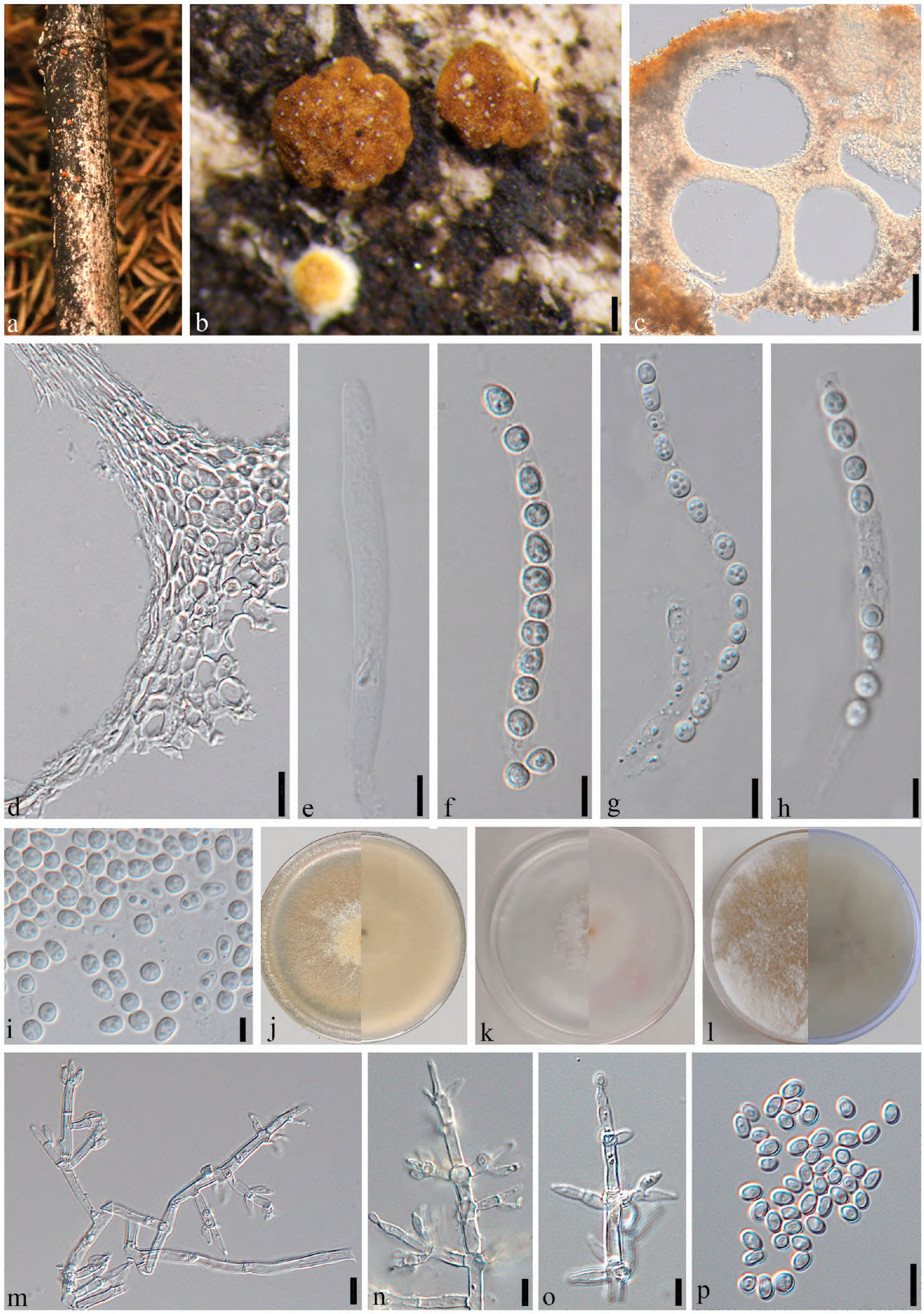

Figure 3. *Trichoderma bashania* (SICAU 25-0179, holotype). **a.** Appearance of ascomata on dead host; **b.** Stromata; **c.** Ascomatal tissue in section; **d.** Peridium; **e–h.** Asci; **i.** Ascospores; **j.** Culture on PDA (15 days at 30 °C); **k.** Cultures on SNA (15 days at 30 °C); **l.** Cultures on MEA (15 days at 30 °C); **m–o.** Conidiophores and phialides (PDA 30 days at 30 °C); **p.** Conidia (PDA 30 days at 30 °C). Scale bars: 500 μm (**b**); 200 μm (**c**); 10 μm (**d–h, p**); 5 μm (**i, m–o**).

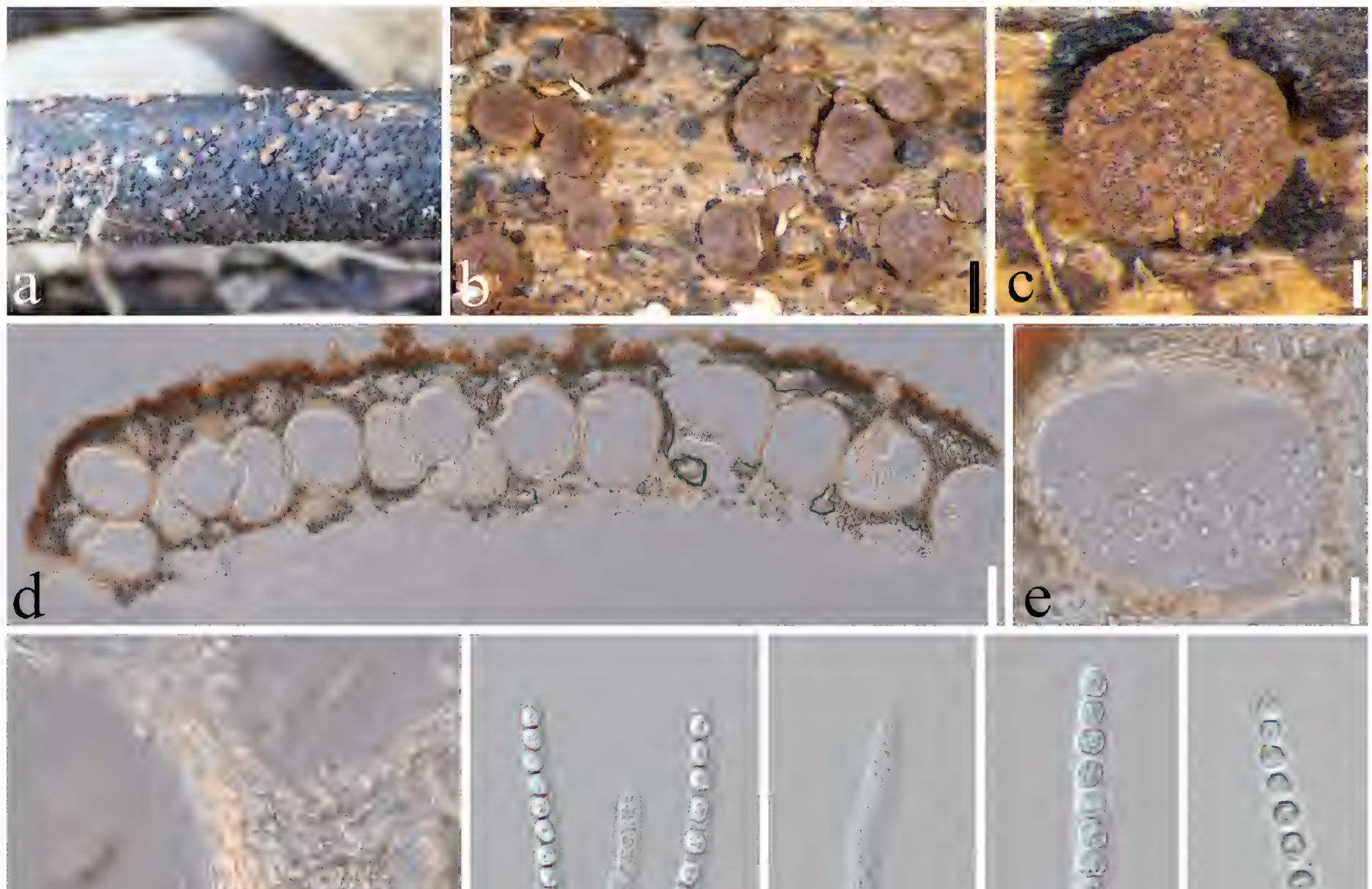

Figure 4. *Trichoderma fargesia* (SICAU 25-0185, holotype). **a.** Appearance of ascomata on dead host; **b, c.** Stromata; **d, e.** Ascomatal tissue in section; **f.** Peridium; **g–j.** Asci; **k, l.** Ascospores. Scale bars: 5 mm (**b**); 1 mm (**c**); 200 µm (**d**); 50 µm (**e**); 15 µm (**f–j**); 5 µm (**k, l**).

with diameters of 2–8 mm and thicknesses of 1–3 mm (n = 20). Young stromata surface velvety, covered pale yellowish hairs. Later stages, surface finely granular from perithecial contours, appearing without any covering. Ostiolar dots on stromata inconspicuous. ***Ascomata*** nearly spherical and densely arranged, measuring

150–280 × 90–240 μm ($\bar{x}$ = 220 × 175 μm, n = 20). ***Ostioles*** flush with the stroma surface, with apical widths of 34–50 μm and heights of 45–78 μm (n = 20). ***Peridium*** ranging from hyaline to brown, lateral thickness of 4–14 μm and a basal thickness of 8–18 μm (n = 20). ***Asci*** cylindrical, measuring 65–84 × 4–6 μm ($\bar{x}$ = 75 × 5 μm, n = 30), inclusive of a stipe 7–12 μm long, containing 16-ascospores, apex not thickened, hyaline, cylindrical. ***Ascospores*** 3–5 × 2.5–4 μm ($\bar{x}$ = 4.5 × 2.8 μm, n = 40) hyaline, nearly spherical to ellipsoidal. ***Asexual morph***: Not observed.

Material examined. China • Shaanxi Province, Ankang City, Ningshan, Primitive Forest of *Fargesia qinlingensis* (33°35'45.67"N, 108°3'54.18"E, Alt. 2280 m), 15 October 2024, Feihu Wang, WFH202410014, (SICAU 25-0185, holotype). *ibid*. WFH202410014B (SICAU 25-0186, paratype).

GenBank accession numbers. SICAU 25-0185 (ITS: PV789475; *tef*1-α: PV828328; *rpb*2: PV828320); SICAU 25-0186 (ITS: PV789476; *tef*1-α: PV828329; *rpb*2: PV828321).

Culture characters. No germination was observed on potato dextrose agar (PDA), oatmeal agar (SNA), or malt extract agar (MEA).

Notes. Phylogenetically, *Trichoderma fargesia* strain SICAU 25-0185 and SICAU 25-0186) formed a distinct clade and is related to *T. parahamatum* (GDMCC 3.1020) in the Koningii clade, but the similarities of *rpb*2 and *tef*1-α between these two species were only 86.2% and 90.5%, respectively. During the isolation and cultivation process, *T. fargesia* failed to germinate in sterile water and several culture media. *Trichoderma parahamatum* has only been described in its asexual stage, characterized by conidiophores that are coiled, undulate, or hamate, bearing phialides that are ampulliform to subglobose, short, and wide. Conidia are subglobose, ellipsoidal to breviter cylindracea, and green. Moreover, *T. parahamatum* is capable of germinating in several culture media. Phylogenetic analysis indicates that the new taxon *T. fargesia* (SICAU 25-0185) is closely related to *T. parahamatum* (GDMCC 3.1020) (Fig. 1). However, our strain exhibits nucleotide differences from *T. parahamatum* in the *rpb*2 region amounting to 13.8% (155/1116, 8 gaps), 9.5% (86/898, 6 gaps) differences in *tef*1-α. Pairwise nucleotide comparisons further support the distinction of *T. fargesia* from related taxa. The PHI test revealed no significant recombination event between our strain and the closely related taxa (Φw = 1.00) (Fig. 2). *Trichoderma fargesia* forms a distinct branch within the Koningii clade, leading to its identification as a new species.

Trichoderma mianyangensis **Feihu Wang & C.L. Yang, sp. nov.**

Index Fungorum number: IF904042

Fig. 5

Etymology. The specific epithet is derived from the collection locality of the type specimen, Mianyang City, Sichuan Province.

Holotype. SICAU 25-0183.

Habitat. On the culm of *Phyllostachys sulphurea*.

Description. ***Sexual morph***: ***Stromata*** solitary, scattered, or aggregated in small numbers (2–3), presenting a pulvinate, discoid, Irregular in shape, broad-based in attachment. Ranging in color from light yellow to nearly citrine, with

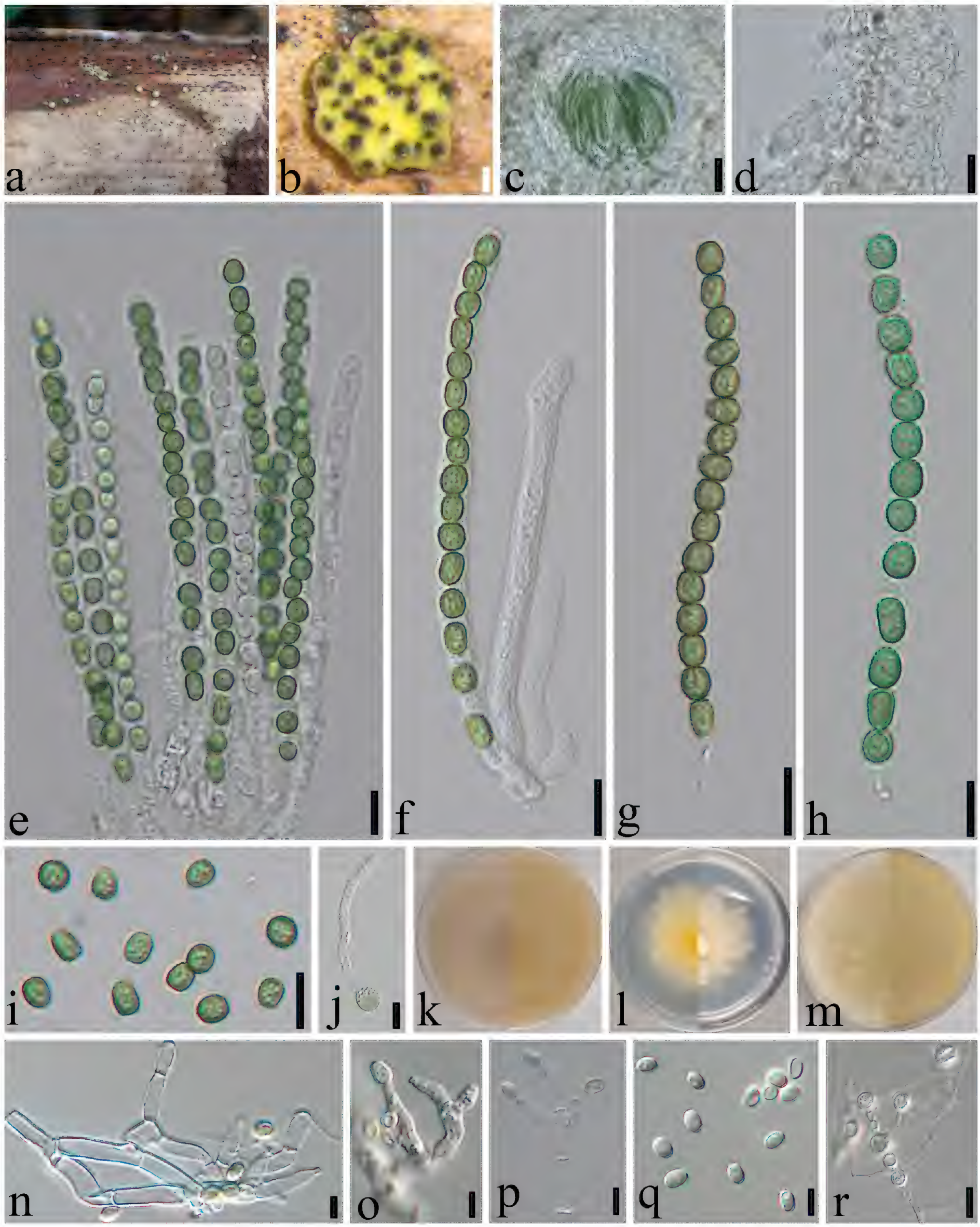

Figure 5. *Trichoderma mianyangensis* (SICAU 25-0183, holotype). **a.** Appearance of ascomata on dead host; **b.** Stromata; **c.** Ascomatal tissue in section; **d.** Peridium; **e–h.** Asci; **i.** Ascospores; **j.** Germinated ascospore; **k.** Culture on PDA (15 days at 25 °C); **l.** Cultures on SNA (15 days at 25 °C); **m.** Cultures on MEA (15 days at 25 °C); **n–p.** Conidiophores and phialides (PDA 30 days at 25 °C); **q.** Conidia (PDA 30 days at 25 °C); **r.** Chlamydospores (PDA 30 days at 25 °C). Scale bars: 500 μm (**b**); 50 μm (**c**); 10 μm (**e–i, r**); 5 μm (**j, n–q**).

diameters of 3–10 mm and thicknesses of 1–3 mm (n = 30). Surface finely tuberculate or wrinkled. Margins rounded, angular, or undulate, typically free, sides slightly retracted inward. Ostiolar dots minute, distinct, surface rarely convex. ***Ascomata*** nearly spherical, densely arranged, and numerous, measuring 220–360 × 180–270 µm ($\bar{x}$ = 290 × 170 µm, n = 20). ***Ostioles*** flush with the surface, with apical widths of 35–58 µm and heights of 40–78 µm (n = 20). ***Peridium*** hyaline to light yellow, with a lateral thickness of 8–12 µm and a basal thickness of 11–17 µm (n = 20). ***Asci*** 82–108 × 5–7 µm ($\bar{x}$ = 100 × 6 µm, n = 20), inclusive of a stipe 9–20 µm long, cylindrical, containing 16-ascospores, apex slightly thickened, hyaline. ***Ascospores*** 3–6 × 3.5–4.5 µm ($\bar{x}$ = 5 × 4 µm, n = 40), green, partially hyaline, finely spinulose, and ranging from nearly spherical to slightly ovoid in shape. ***Asexual morph***: ***Conidiophores*** consist of an erect stipe with a 1–3 celled branch at the apex. ***Phialide*** t lageniform, ampulliform, or subglobose, measuring 4–12 × 1.5–3 µm ($\bar{x}$ = 9 × 2 µm, n = 30). ***Conidia*** hyaline, ellipsoidal to oblong, less commonly subglobose, smooth, and measure 2.5–4 × 1.5–3 µm ($\bar{x}$ = 3 × 2 µm, n = 40).

Material examined. China • Sichuan Province, Mianyang City, Pingwu, Primitive Forest of *Phyllostachys sulphurea* (32°37'13.81"N, 104°31'21.63"E, Alt. 1363 m), 18 October 2024, Feihu Wang, WFH20240092, (SICAU 25-0183, holotype), ex-type culture SICAUCC 25-0155. *ibid*. WFH20240092B (SICAU 25-0184, paratype), living culture SICAUCC 25-0156.

GenBank accession numbers. SICAUCC 25-0155 (ITS: PV789477; *tef*1-α: PV828330; *rpb2*: PV828322); SICAUCC 25-0156 (ITS: PV789478; *tef*1-α: PV828331; *rpb2*: PV828323)

Culture characters. Three media were tested for fungal growth: potato dextrose agar (PDA), oatmeal agar (SNA), and malt extract agar (MEA), all incubated at the optimal temperature of 25 °C. On PDA, the mycelium fully covered a 60-mm Petri dish within 8–10 days, forming a circular colony with a regular margin and velvety to floccose surface hyphae arranged radially. Abundant aerial hyphae extended toward the margin, becoming floccose post-conidiation. By day 12, conidiation initiated around the inoculation plug, forming small white clusters that expanded peripherally while remaining white. No diffusing pigment or distinct odor was detected. On SNA, growth was slower, requiring 20 days to fully colonize the dish (colony diameter: 20 mm after 12 days). The colony remained white and transparent, with sparse mycelium on the agar surface. Central mycelia became hollow, forming a loose, white film-like structure. No pigment or odor was observed, though the inoculation site gradually greened, conidia were absent. On MEA, rapid growth covered the dish within 7–9 days, yielding a circular, transparent colony with conspicuous variation in hyphal width (no zonation). Aerial hyphae were sparse initially but thickened at distal/lateral margins over time. By day 11, conidiation initiated on aerial hyphae near the proximal margin, forming fluffy tufts that compacted into 1–3 mm pustules with, pustules remained white for 5–8 days, then turned brown. No diffusing pigment or odor was detected.

Notes. Phylogenetically, *Trichoderma mianyangensis* strain SICAU 25-0183 and SICAU 25-0184 formed a distinct clade and is related to *T. parahongkuii* (GDMCC 3.1019) in the Koningii clade, but the similarities of *rpb*2 and *tef*1-α between these two species were only 94.4% and 80.9%, respectively. From a morphological perspective, our newly described taxon, *Trichoderma mianyangensis*,

shares some common characteristics with *T. parahongkuii* isolated from soil in a *Chimonanthus praecox* orchard. There are certain differences in cultural characteristics between *T. mianyangensis* and *T. parahongkuii*, differences in cultural characteristics exist between *T. mianyangensis* and *T. parahongkuii*. *Trichoderma mianyangensis* lacks any odor, while *T. parahongkuii* emits a noticeable fruity scent, and the conidia of *T. mianyangensis* are smaller (2.5–4 × 1.5–3 µm vs. 3.6–5.1 × 2.6–3.5 µm) (Zhao et al. 2025). Phylogenetic analysis reveals that the new taxon, *T. mianyangensis* (SICAU 25-0183), is closely related to *T. parahongkuii* (GDMCC 3.1019), with strong statistical support (Fig. 1). However, our strain differs from *T. parahongkuii* in the *rpb*2 region amounting to 5.6% (63/1130, 0 gap), 19.1% (178/930, 61 gaps) differences in *tef*1-α. Pairwise nucleotide comparisons further support the distinction of *T. mianyangensis*. The PHI test revealed no significant recombination event between our strain and the closely related taxa (Φw = 1.00) (Fig. 2). These differences also support the classification of *T. mianyangensis* as a distinct species.

Trichoderma yaanensis **Feihu Wang & C.L. Yang, sp. nov.**

Index Fungorum number: IF904044

Fig. 6

Etymology. The specific epithet is derived from the collection locality of the type specimen, Ya'an City, Sichuan Province.

Holotype. SICAU 25-0181.

Habitat. On the culm of *Bambusa emeiensis*.

Description. ***Sexual morph***: Not observed. ***Asexual morph***: ***Conidiophores*** are short and simple, either erect or arising obliquely from the surface hyphae. ***Phialides*** are solitary, whorls, and often borne on one-celled lateral branches, being conical or cylindrical in shape. ***Conidia*** 3.5–5.5 × 2–3.5 µm ($\bar{x}$ = 4 × 2.6 µm, n = 40), pale green, smooth, ovoid or ellipsoidal.

Material examined. China • Sichuan Province, Ya'an City, Yucheng, *Bambusa emeiensis* industrial base (29°52'44.35"N, 103°5'23.37"E, Alt. 871 m), 28 April 2024, Feihu Wang, WFH202404030, (SICAU 25-0181, holotype), ex-type culture SICAUCC 25-0153. *ibid*. WFH202404030B (SICAU 25-0182, paratype), living culture SICAUCC 25-0154.

GenBank accession numbers. SICAUCC 25-0153 (ITS: PV789473; *tef*1-α: PV828326; *rpb*2: PV828318); SICAUCC 25-0154 (ITS: PV789474; *tef*1-α: PV828327; *rpb*2: PV828319).

Culture characters. Three media were tested for fungal growth: potato dextrose agar (PDA), oatmeal agar (SNA), and malt extract agar (MEA), all incubated at the optimal temperature of 25 °C. On PDA, the mycelium fully covered a 60 mm Petri dish within 9–10 days, forming a circular, dense colony with a regular margin and sparse surface hyphae arranged radially. By day 15, small white velvety masses appeared, later browning and producing conidia; no odor or pigment was detected. On SNA, growth was slower, requiring 15 days to cover the dish. The colony remained white with sparse aerial hyphae and no zonation, though the inoculation site gradually greened, conidia and pigments were absent. On MEA, rapid growth covered the dish in 8 days, yielding a rough-edged colony with abundant aerial hyphae that thickened at distal/lateral

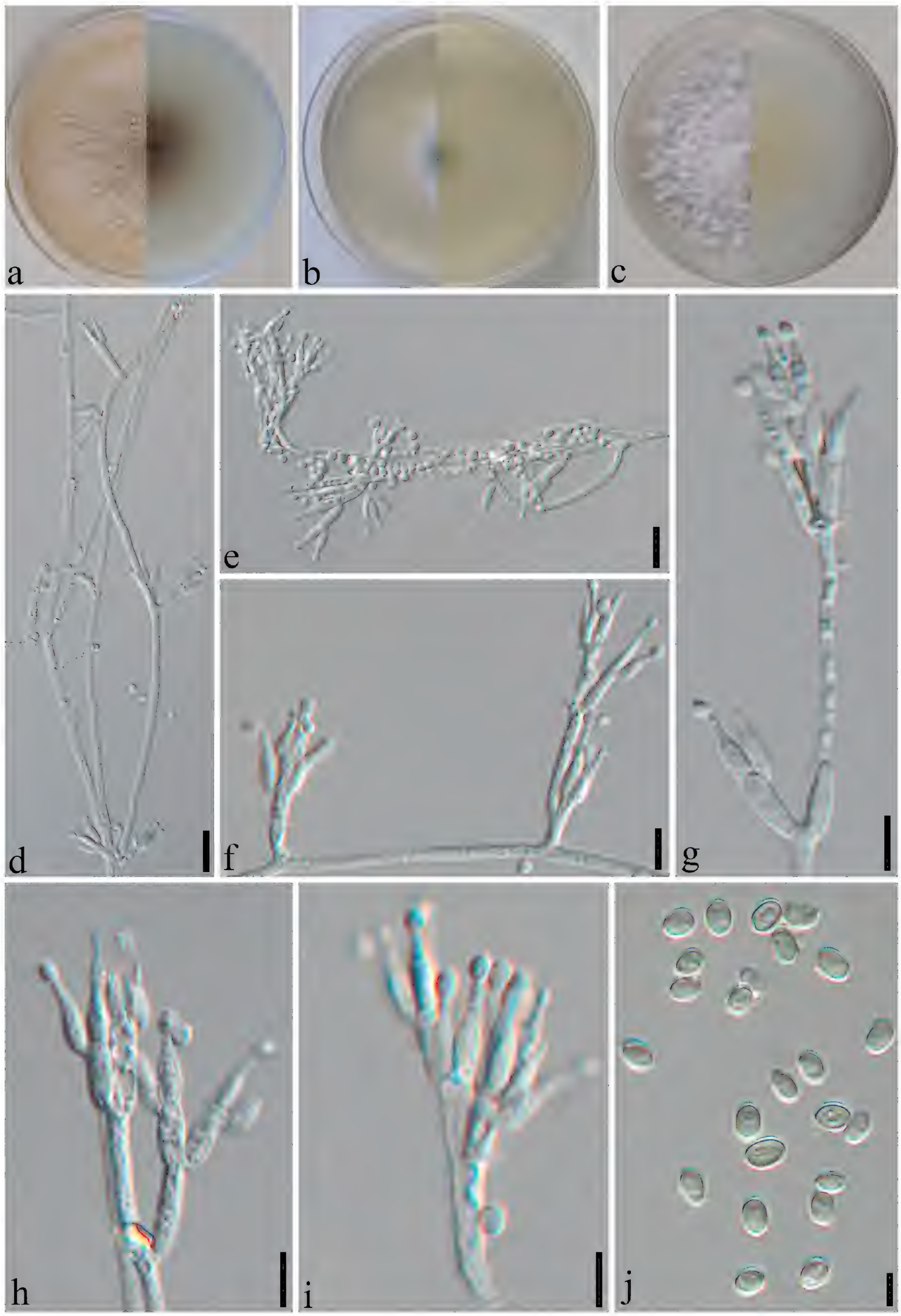

Figure 6. *Trichoderma yaanensis* (SICAU 25-0181, holotype). **a.** Culture on PDA (15 days); **b.** Cultures on SNA (15 days at 25 °C); **c.** Cultures on MEA (15 days at 25 °C); **d–i.** Conidiophores and phialides (PDA 30 days at 25 °C); **j.** Conidia (PDA 30 days at 25 °C). Scale bars: 20 μm (**d, e**); 15 μm (**f**); 10 μm (**g–i**); 5 μm (**j**).

margins. Sporulation initiated after 10 days, forming fan-shaped patterns, no odor or pigment was observed.

Notes. Phylogenetically, *Trichoderma yaanensis* strain SICAU 25-0181 and strain SICAU 25-0182 formed a distinct clade and is related to *T. merleae* (MST FP3586) in the Koningii clade, but the similarities of *rpb*2 and *tef*1-α between these two species were only 97.9% and 99.5%, respectively. *Trichoderma yaanensis* is classified within the Koningii clade and is phylogenetically closely related to *T. merleae*. Currently, there is no available morphological description for *T. merleae*, only molecular data have been documented, thereby rendering morphological comparisons infeasible. Phylogenetic analysis reveals that the new taxon, *Trichoderma yaanensis* (SICAU 25-0181), is closely related to *T. merleae* (MST FP3586), with strong statistical support (Fig. 1). However, our strain differs from *T. merleae* in the ITS region amounting to 11.7% (67/572, 32 gap), 2.1% (17/807, 0 gap) differences in *rpb*2, 0.5% (5/921, 0 gap) differences in *tef*1-α. Pairwise nucleotide comparisons further support the distinction of *T. yaanensis*. The PHI test revealed no significant recombination event between our strain and the closely related taxa (Φw = 1.00) (Fig. 2). Therefore, based on the phylogenetic differences, *T. yaanensis* is introduced as a new species within *Trichoderma*.

Discussion

Based on ITS, *tef*1-α, and *rpb*2 DNA sequence datasets as well as morphological evidence, this study describes four new species within the genus *Trichoderma*, classifying them as *Trichoderma bashania*, *T. fargesia*, *T. mianyangensis* and *T. yaanensis*. Phylogenetic analysis revealed their distinct genetic relationships and positions within the family *Trichoderma*. Species of this genus are widely distributed globally, and have been reported in various plants, soil, water, as well as in the air. (Liu et al. 2012; Li et al. 2013; Jambhulkar et al. 2024). The discovery of these new species is significant for understanding the species diversity, classification, and geographical distribution of *Trichoderma*.

Bamboo holds extremely high economic, ecological, and cultural values, and the bamboo species that serve as the staple food for giant pandas are imbued with even more unique significance. China is the only country in the world that has wild giant pandas, and the bamboo species that constitute their primary diet are exclusively distributed within this region. The staple bamboo species that giant pandas rely on for food are facing severe challenges, which will lead to food shortages for giant pandas and further accelerate their extinction. Currently, the staple bamboo species for giant pandas are confronted with numerous threats, including global warming, human-induced harvesting of bamboo shoots, grazing, infrastructure development, as well as pests and diseases (Liu et al. 2014; Xiang et al. 2024). Fungi play a crucial role in the ecosystem of the staple bamboo species for giant pandas. They facilitate the growth of the staple bamboo, enhance its resistance to adverse conditions, and participate in the nutrient cycling beneath the bamboo forest. Through mechanisms such as decomposing organic matter, providing nutrients, and promoting the development of bamboo roots, fungi indirectly influence the growth and quality of the bamboo, thereby affecting the food source for giant pandas. In contrast, pathogenic fungi can directly cause the death of the staple bamboo. Research demonstrates that when *Trichoderma* colonizes bamboo as an endophytic fungus, it substantially enhances the plant's

resistance mechanisms. Following bamboo senescence, this versatile fungus transitions to a saprophytic lifestyle, efficiently decomposing lignocellulosic residues and facilitating nutrient cycling within bamboo ecosystems (Bhunjun et al. 2024). Therefore, *Trichoderma* plays a crucial role in the bamboo forest ecosystem. Currently, research on fungi associated with the staple bamboo species for giant pandas is still remains largely unexplored. Therefore, conducting extensive surveys and sampling will contribute to enriching the fungal resources associated with the staple bamboo species for giant pandas.

Among the four newly discovered species, three originate from Sichuan. This phenomenon is primarily due to the fact that the staple bamboo species for giant pandas are predominantly distributed in this region. The increasing discovery of fungal species offers new clues for exploring the biodiversity of this area and also provides crucial information for the conservation of its ecosystems. All these species are distributed within the Koningii clade, a branch that encompasses multiple economically significant species (Han et al. 2023; Xiang et al. 2024). Some of these species are important pathogens, while others have been developed into biological agents. Therefore, further research on these species is necessary.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Use of AI

No use of AI was reported.

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Author contributions

Conceptualization: Feihu Wang, Yinggao Liu, and Chunlin Yang; Data curation: Feihu Wang, Xiulan Xu, and Feng Liu; Funding acquisition:Yinggao Liu and Chunlin Yang; Investigation: Feihu Wang; Project administration: Yinggao Liu; Resources: Yinggao Liu and Chunlin Yang; Supervision: Yinggao Liu and Chunlin Yang; Writing––original draft: Feihu Wang; Writing—review and editing: Feihu Wang, Shasha Xiang, Xinyue Li, Yinggao Liu, and Chunlin Yang. All authors have read and agreed to the published version of the manuscript.

Author ORCIDs

Xiulan Xu https://orcid.org/0000-0002-6832-5421
Feng Liu https://orcid.org/0000-0003-4580-7169
Chunlin Yang https://orcid.org/0000-0002-3854-8835

Data availability

All of the data that support the findings of this study are available in the main text.

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